# NUMB phosphorylation destabilizes p53 and promotes self-renewal of tumor-initiating cells by a NANOG-dependent mechanism in liver cancer.

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**Public Summary:**
Human stem cell populations are maintained through self-renewing divisions in which one daughter cell commits to a particular fate while the other retains the parental traits. The NUMB, a tumor suppressor, in conjunctions with another tumor suppressor protein p53, preserves this property and acts as a barrier against deregulated expansion of Tumor-associated stem cells.As the molecular mechanism governing the stability of the NUMB-p53 interaction are poorly understood, we tried to identify the molecule/s govern this process. Using cancer cell lines, tumor-initiating cells (TICs) of liver, the mouse model, and clinical human samples, we identified that phosphorylations of NUMB destabilize p53 and promotes proliferation (self-renewal) of TICs by transcription factor NANOG-dependent manner. NANOG phosphorylates NUMB via aPKCζ, through the direct induction of Aurora A kinase and the repression of an aPKCζ inhibitor, LGL-2. Phosphorylation of NUMB by aPKCζ destabilizes the NUMB-p53 interaction, p53 proteolysis and to deregulate proliferation in TICs. Our work identifies the NANOG-NUMB-p53 signaling axis is an important regulatory pathway in liver tumorigenesis and suggest a therapeutic strategy by targeting NUMB-phosphorylation.

**Scientific Abstract:**
Stem cell populations are maintained through self-renewing divisions in which one daughter cell commits to a particular fate whereas the other retains the multipotent characteristics of its parent. The NUMB, a tumor suppressor, in conjunction with another tumor-suppressor protein, p53, preserves this property and acts as a barrier against deregulated expansion of tumor-associated stem cells. In this context, NUMB-p53 interaction plays a crucial role to maintain the proper homeostasis of both stem cells, as well as differentiated cells. Because the molecular mechanism governing the assembly and stability of the NUMB-p53 interaction/complex are poorly understood, we tried to identify the molecule(s) that govern this process. Using cancer cell lines, tumor-initiating cells (TICs) of liver, the mouse model, and clinical samples, we identified that phosphorylations of NUMB destabilize p53 and promote self-renewal of TICs in a pluripotency-associated transcription factor NANOG-dependent manner. NANOG phosphorylates NUMB by atypical protein kinase C zeta (aPKCzeta), through the direct induction of Aurora A kinase (AURKA) and the repression of an aPKCzeta inhibitor, lethal (2) giant larvae. By radioactivity-based kinase activity assays, we showed that NANOG enhances kinase activities of both AURKA and aPKCzeta, an important upstream process for NUMB phosphorylation. Phosphorylation of NUMB by aPKCzeta destabilizes the NUMB-p53 interaction and p53 proteolysis and deregulates self-renewal in TICs. CONCLUSION: Post-translational modification of NUMB by the NANOG-AURKA-aPKCzeta pathway is an important event in TIC self-renewal and tumorigenesis. Hence, the NANOG-NUMB-p53 signaling axis is an important regulatory pathway for TIC events in TIC self-renewal and liver tumorigenesis, suggesting a therapeutic strategy by targeting NUMB phosphorylation. Further in-depth in vivo and clinical studies are warranted to verify this suggestion.